APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

REMARKS

Upon entry of the present amendments, claims 1-5, 11 and 14 are pending.

Applicants have amended claims 1-5, 11 and 14.

The amendments add no new matter.

The Examiner has suggested that applicant amend the first line of the specification to update the status of the priority documents. Applicants have amended the specification to include this information.

The Examiner has objected to the specification because of the recitation of "." at the beginning of a sentence throughout the specification. The specification has been amended to substitute a "-" in place of a "." in each instance throughout the specification.

The Examiner has objected to the phrase "N0-Tyr-BSA" on page 54, line 5 of the specification. As suggested by the Examiner, this has been corrected to recite "NO-Tyr-BSA".

The Examiner has objected to the "et" on page 43, line 10. As suggested by the Examiner, this has been corrected to recite "and".

For the Examiner's convenience, a substitute and corrected specification is submitted herewith which incorporates all of the above amendments to the specification and also includes a description of the figures submitted herewith.

Drawings

Figures 1-29 as originally recited in the specification are enclosed herewith. Applicant's note that the figures were originally part of the international application which was transmitted by the International Bureau.

35 USC § 112, second paragraph

The Examiner has rejected claims 2, 4-5, 11 and 14 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. The Examiner states that the phrase "characterized by the fact" in claims 2, 4-5, 11 and 14 is unclear. Applicants have amended claims 2, 4-5, 11 and 14 to remove the phrase "characterized by the fact", and have substituted the term "wherein" in place thereof.

The Examiner also recites that the phrase "A pharmaceutical compound" in claim 11 is ambiguous and one of ordinary skill in the art cannot appraise the metes and bounds of the claimed invention. Claim 11 has been amended to delete the term "compound", and the term "composition" has been substituted in place thereof.

Applicants have also amended the form of the claims, so that each claim is the object of a sentence starting with "We claim."

35 USC § 102

The Examiner has rejected claims 1-4 under 35 U.S.C. § 102(b) as allegedly anticipated by Boullerne *et al.*, J. of Neuroimmunology 60: 117-124 (1995) ("*Boullerne*"). Applicants respectfully traverse.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. MPEP § 2131. "There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ 2d 1001, 1010 (Fed. Cir. 1991). Here, *Boullerne* does not disclose the claimed invention.

Boullerne does not anticipate amended claims 1-4. As amended, claim 1 is directed to a **purified antibody** wherein the antibody binds specifically to a nitrosylated protein. *Boullerne* concerns the nitrosation of amino acid conjugates and the use of NO-amino acid-g-BSA conjugates to coat a plate for an enzyme-linked immunosorbent assay (ELISA) experiment testing multiple sclerosis sera. *Boullerne* disclosed that a significant antibody reaction against the S-nitroso-cysteine was observed suggesting the presence in the sera of antibodies recognizing this neo-antigen. *Boullerne* does not provide any teaching directed to the use of a **purified antibody** and does not disclose a **purified antibody** wherein the antibody binds specifically to a nitrosylated protein. *Boullerne* recites only that the antibodies simply recognize nitrosylated proteins. In the present application, the procedure for purifying the recited antibodies is described in the specification on pages 25-26.

Accordingly, *Boullerne* does not recite each and every element of amended claim 1. Claims 2-4 depend directly from amended claim 1 and thus incorporate all the limitations of

amended claim 1 therein. For the same reasons stated with regard to amended claim 1, claims 2-4 are not anticipated by *Boullerne*. Consequently, claims 1-4 are novel over *Boullerne*. Applicants submit that amended claims 1-4 are allowable and respectfully request reconsideration and withdrawal of the present rejection.

35 U.S.C. §103

The Examiner has rejected claims 1-3, 5 and 11 under 35 U.S.C. § 103(a) as being unpatentable over Stamler *et al.*, Proc. Natl. Acad. Sci. USA 89: 444-448 (1992) ("*Stamler*") or U.S. Pat. No. 5,583,101 to Stamler *et al.* ("the '101 patent"), each in view of Campbell *et al.*, Monoclonal Antibody Technology, Elsevier Science Publishers (1984)("*Campbell*"). Applicants respectfully traverse.

It is well recognized under U.S. law, that any rejection of a claim for obviousness over a combination of prior art references must establish that: (1) the combination produces the claimed invention; and (2) the prior art contains a suggestion or motivation to combine the prior art references in such a way as to achieve the claimed invention. (*In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). In addition, the Examiner's prima facie case must include a finding that one of ordinary skill in the art at the time the invention was made would have reasonably expected the claimed invention to work. (*See In re O'Farrell*, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988); *In re Dow Chem.*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Stamler teaches that proteins can be S-nitrosylated and that S-nitrosylation of proteins endows these molecules with potent and long-lasting endothelium-derived relaxation factor (EDRF)-like effects of vasodilation and platelet inhibition that are mediated by guanylate cyclase activation. *Stamler* also teaches methods for preparing S-nitroso proteins.

The '101 patent is directed to the use of nitrogen oxide species and adducts to inhibit skeletal muscle contraction, particularly by administering a skeletal muscle relaxing amount of a compound selected from the group consisting of nitroxyl ion (NO^-), nitrosonium ion (NO^+), nitric oxide and nitric oxide adducts or providers.

Campbell is a generalized reference directed to a discussion of the general properties and applications of **monoclonal antibodies** compared with antiserum. With regard to the purity of mononclonal antibodies, *Campbell* discusses only that bovine immunoglobulin obtained from foetal calf serum may have contaminating proteins contained therein that may be removed by affinity chromatography.

The Examiner notes that neither *Stamler* nor the '101 patent teach antibodies to nitrosylated proteins. Based on a single recitation in *Campbell* which states that it is customary to both clone genes and make antibodies to it, the Examiner asserts that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make antibodies specific for nitrosylated protein comprising a transporter of NO coupled to a carrier protein such as albumin via a coupling agent such as glutaraldehyde. The Examiner proceeds to state that one would have been motivated, with a reasonable expectation of success, to generate monoclonal antibodies to nitrosylated protein based on the fact that it is a conventional practice in the art to do so for further study, characterization, detection and diagnostic assays.

Even if the way to obtain S-nitrosoprotein is known as taught in *Stamler*, and even if techniques are well-known to produce antibodies, such as described *Campbell*, it cannot be said that one skilled in the art would deem it obvious from a combination of these two references to produce **purified antibodies** which recognize and specifically bind to a nitrosylated protein as

recited in amended claim 1. One skilled in the art is faced with several obstacles to obtain such **purified antibodies** capable of recognizing *in vivo* S-nitrosoproteins, and which neutralize their properties. For example, to obtain anti-nitroso-cysteine antibodies as described in the present application, the Applicant's had to conjugate the cysteine according to a process in which the cysteine is an immunogen and the NO is an immunodominant element of the antigen. Moreover, in the monoclonal approach as described on pages 37-43 of the specification of the instant application, to stimulate a sufficient population of lymphocytic clones for hybridization, particular methods of administration of the immunogen were used to obtain an immunogenic response directed to NO-cysteine. The lymphocytic hybridization and the monoclonal selection require knowledge and know-how not taught in any of the cited prior art references.

Thus, neither *Stamler* nor the '101 patent, alone or in combination with *Campbell* teach or suggest a **purified antibody** wherein the antibody binds specifically to a nitrosylated protein. Furthermore, neither *Stamler* nor the '101 patent, alone or in combination with *Campbell* teach or suggest a purified polyclonal or monoclonal antibody. Based on the disclosures of *Stamler* and the '101 patent, one skilled in the art would not be motivated to combine either of these references with the teaching of *Campbell* to provide **a purified antibody wherein the antibody binds specifically to a nitrosylated protein** as recited in amended claim 1. There is no explicit nor implicit suggestion or motivation to combine the teachings of *Stamler* or the '101 patent with the teaching of *Campbell* so as to make the precise choices necessary to arrive at the purified antibody of amended claim 1. Amended claim 1 is thus not obvious. Claims 2, 3 and 5 depend directly from claim 1 and incorporate all the limitations of amended claim 1 therein. As such,

claims 2, 3 and 5 are likewise not obvious in view of the cited prior art for the same reasons as recited with regard to amended claim 1.

Claim 11 has been amended to recite a pharmaceutical composition comprising: (a) a **purified antibody** that binds specifically to a nitrosylated protein; and (b) a pharmaceutically acceptable vehicle. While the '101 patent teaches a pharmaceutical composition, it is directed to a pharmaceutical composition of a skeletal muscle relaxing amount of a compound selected from the group consisting of nitroxyl ion (NO^-), nitrosonium ion (NO^+), nitric oxide and nitric oxide adducts or providers, along with a pharmaceutically acceptable carrier. There is no teaching or suggestion in the '101 patent, alone or in combination with *Stamler* or *Campbell* directed to the pharmaceutical composition recited in amended claim 11.

In view of the foregoing amendments and arguments, it is respectfully submitted claims 1-3, 5 and 11 are not obvious in view of the references cited by the Examiner, and that the present rejections under 35 U.S.C. §103(a) should be withdrawn

The Examiner has rejected claim 14 under 35 U.S.C. § 103(a) as being unpatentable over Stamler *et al.*, Proc. Natl. Acad. Sci. USA 89: 444-448 (1992) ("*Stamler*") or U.S. Pat. No. 5,583,101 to Stamler *et al.* ("the '101 patent"), each in view of Campbell *et al.*, Monoclonal Antibody Technology, Elsevier Science Publishers (1984)("*Campbell*") as applied to claims 1-3, 5 and 11, and further in view of U.S. Pat. No. 5,858,682 to Gruenwald *et al.* ("the '682 patent"). Applicants respectfully traverse.

The Examiner recites that the claimed invention in claim 14 differs from the references only by the recitation of a kit comprising antibody for detection of any nitrosylated proteins in a

biological specimen. The Examiner states that the '682 patent teaches a kit comprising antibody for diagnostic (see col. 3, line 40; col. 6, line 17; col. 8, line 36, in particular.

The *Stamler* and *Campbell* references and the '101 patent were described and discussed infra with regard to the rejection to claims 1-3, 5 and 11.

The '682 patent discloses a monoclonal antibody which binds specifically with an E2A/pbx1 fusion epitope. Contrary to the Examiner's assertion, the '682 patent discloses in col. 3, lines 46, that optimal panels of mAbs(E2A/pbx1 junction and pbx1) for each application will be selected. According to the '682 patent, these will serve as basis for a **clinical kit** development for childhood pre-B leukemia. Such a **diagnostic kit** will allow clinical reference laboratories to screen a heterogeneous population of cells from blood or bone marrow for the presence of the E2A/pbx1 fusion protein resulting in the fast and reliable detection of t(1;19) bearing leukemia cells. In col. 8, lines 36-41, the '682 patent recites that a "**diagnostic system in kit form** of the present invention includes, in an amount sufficient for at least one assay, a polypeptide, polypeptide admixture, antibody composition or monoclonal antibody composition of the present invention, as a packaged reagent. Instructions for use of the packaged reagent are also typically included." The diagnostic system of the '682 patent may further include a binding agent. The '682 patent teaches that the polypeptide, antibody molecule composition or monoclonal antibody molecule composition of the invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

Amended claim 14 recites a kit for ***in vitro* detection of nitrosylated proteins in a biological specimen** comprising (a) a purified antibody that binds specifically to a ntirosylated

protein and (b) reagents to produce a medium favorable for an immunological reaction between said antibody and any nitrosylated proteins that may be present in a biological specimen.

As recited, the '682 teaches a "**diagnostic system in kit form**" for performing an assay. The '682 patent also teaches that the ingredients of its diagnostic system are affixed on a solid support. The kit recited in amended claim 14 of the instant application is directed to the ***in vitro* detection** of nitrosylated proteins in a biological specimen. There is thus no teaching or suggestion in the '682 patent directed to a kit for *in vitro* detection of nitrosylated proteins in a biological specimen. Nor does the '682 patent teach or suggest a kit which comprises a purified antibody that binds to specifically to a nitrosylated protein, and reagents to produce a medium favorable for an immunological reaction between the purified antibody and any nitrosylated proteins that may be present in a biological specimen. The '682 patent provides no teaching or suggestion directed to producing a medium favorable for an immunological reaction between the purified antibody and any nitrosylated proteins that may be present in a biological specimen in a kit as recited in amended claim 14. Accordingly, there is no motivation or teaching to suggest to one of skill in the art to combine the '682 patent with the teachings of *Stamler*, or the '101 patent, each in view of *Campbell*, and further in view of the '682 patent, in order to arrive at the kit as recited in amended claim 14.

In view of the foregoing amendments and arguments, it is respectfully submitted that the present claims are not obvious in view of the references cited by the Examiner. Applicants respectfully request reconsideration and withdrawal of the present rejection.

APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

CONCLUSION

On the basis of the foregoing amendments, the points and concerns raised by the Examiner having been addressed in full, Applicants respectfully submit that the pending claims are in condition for allowance, which action is respectfully requested.

If, upon receipt and review of this amendment, the Examiner believes that the present application is not in condition for allowance and that changes can be suggested which would place the claims in allowable form, the Examiner is respectfully requested to call Applicant's undersigned counsel at the number provided below.

Respectfully submitted,

Ivor R. Elrifi, Reg. No. 39,529
Michel Moreney, Limited Recognition
Barry J. Marenberg, 40,715
Attorneys for Applicants
c/o MINTZ LEVIN
One Financial Center
Boston, Massachusetts 02111
Telephone: (617) 542-6000
Telefax: (617) 542-2241

APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

On page 1, line 4, please insert the following sentence:

-- CLAIM OF PRIORITY

This application is a 35 U.S.C. 371 national application of PCT/FR97/02412, filed on December 23, 1997. This application also claims priority to French Patent Application FR 96/16207.

TECHNICAL FIELD OF THE INVENTION--.

On pg. 1, line 14, insert --BACKGROUND OF THE INVENTION--.

Paragraph beginning on page 1, line 15, has been amended as follows:

-- Nitric oxide, hereinafter designated also as NO, is described as being the smallest molecule made by the cells. Initially assimilated to [EDRF] endothlium derived relaxing factor (EDRF), it was then recognized as a neuromediator, and is thought to be the first neurotransmitter with retrograde activity, as well as a cytostatic/cytotoxic molecule. Because of its strong reactivity, nitric oxide is capable of reacting with a large number of molecules to form conjugates which have multiple functions and therefore participate in many physiological and pathophysiological processes.--

On pg. 2, line 1 , insert --SUMMARY OF THE INVENTION--.

On page 2, line 27, insert --DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating the zone of absorbency of NO-Tyr-BSA;

Fig 2 is a graph illustrating the zone of absorbency of NO-Cys-BSA;

Fig. 3 is a graph illustrating response following immunization with NO-Tyr-BSA;

Fig. 4 is a graph illustrating response following immunization with NO-Cys-BSA;

Fig. 5 is a graph illustrating the avidity of conjugated anti-NO-Tyr Ab and conjugated anti-NO=Cys in competition tests;

Fig. 6 illustrates the kinetics of the formation and concentration in NO-Cys-BSA formed in supernatant of the culture of activated macrophages determined at incubation times: 0, 3, 4, 6, 8, 11, 14, 18 and 20 hours using "C" antiserum;

Fig. 7 illustrates inhibition of the cytostatic effect of the BCG macrophages on the *T. musculi in vitro* in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100;

Fig. 8 illustrates the cytostatic effect of supernatants containing NO-BSA from activated macrophages added to normal macrophages containing *T. musculi*. Inhibition of this effect in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100;

Fig. 9 illustrates the spectrometric analysis of the NO-Cys-G-BSA immunogen and its structural homologue Cys-G-BSA based on wavelength;

Fig. 10 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse;

Fig. 11 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse;

Fig. 12 illustrates the avidity and specificity of the anti-NO-Cys-G antibodies in the mouse (IP);

Fig. 13 illustrates represents the avidity and specificity of the anti-NO-Cys-G mouse antibodies (PC);

Fig. 14 illustrates the avidity and specificity of the anti-NO-Cys-G monoclonal Ab;

Fig. 15a is a high-magnification (100X) immunocytochemical marking illustrating anti-NO-Cys-G monoclonal antibody and showing very clear markings (immunoreactivities) in terms of trypanosomes co-cultivated in the presence of the activated macrophages ;

Fig. 15b is a high-magnification (100X) immunocytochemical marking illustrating a much weaker marking obtained in the co-culture of activated macrophages / trypanosomes, in the presence of NMMA (0.5 mM);

Fig. 15c is s a high-magnification (100X) immunocytochemical marking illustrating a total absence of trypanosome marking was obtained when a normal mouse serum was used;

Fig. 15d is a high-magnification (100X) immunocytochemical marking illustrating antibody "C" having an intensity very close to the one for the monoclonal Ab;

Fig. 15e is a high-magnification (100X) immunocytochemical marking illustrating the anti-NO-Tyr ("T") giving a positive marking with an intensity not as high as the two types of antibodies (monoclonal and polyclonal) directed against the epitope NO-Cys;

Fig. 15f is a high-magnification (100X) immunocytochemical marking illustrating the absence of marking in the primary Ab of a normal rabbit;

Fig. 16 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-BSA epitope;

Fig. 17 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on delipidated NO-BSA epitope;

Fig. 18 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-BSA epitope;

Fig. 19 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-G-BSA epitope;

Fig. 20 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-BSA epitope

Fig. 21 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-G-BSA epitope

Fig. 22 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tryp-G-BSA epitope

Fig. 23 illustrates the OD obtained from ELISA tests on the NO-Cys-G-BSA, NO-Tyr-BSA and NO_2-Tyr-BSA conjugates (the control group) and indicating presence of circulating Ab whose rate increases during attacks an ddecreases during remissions;

Fig. 24 illustrates the OD obtained from ELISA tests on anti-NO-Tyr-BSA and anti-NO2-Tyr-BSA conjugates (the aminoguanidine group);

Fig. 25 illustrates the OD obtained from ELISA tests on anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr and showing the changes over time in the anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr responses (the monoclonal antibody group);

Fig. 26 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO_2-Tyr-BSA conjugates (the control group);

Fig. 27 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO_2-Tyr-BSA conjugates (the NIS group);

Fig. 28 illustrates the progression of antibodies directed against anti-NO-Tyr-BSA and anti-NO2-Tyr-BSA conjugates (the aminoguanidine group); and

Fig. 29 illustrates the progression of antibodies directed against anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr conjugates (the monoclonal antibody group).

DETAILED DESCRIPTION OF THE INVENTION--.

Paragraph beginning at page 7, line 25, has been amended as follows:

--Several types of NOS were cloned and classified in two distinct families: NOS termed constitutive (cNOS) or [inductible] <u>inducible</u> NOS (iNOS)--.

Paragraph beginning on page 23, line 7, has been amended as follows:

-- [Le] <u>The</u> coupling relationship is the number of moles of hapten coupled with a mole of protein:--

Paragraph beginning at page 24, line 7 and extending to page 24, line11, has been amended as follows:

-- Concentration (M) in coupled hapten = X mg hap x CPM [après [after]] <u>after</u> / CPM [avant [before]] <u>before</u> x Vol [av [before]] <u>before</u> x PM hap

APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

where X mg is the quantity of hapten used for the coupling; CPM [av] before is the radioactivity before dialysis; CPM [après] after is the radioactivity after dialysis; Vol [av] before is the volume before dialysis; PM hap is the molecular weight of the hapten.--

Paragraph beginning on page 25, line 11, has been amended as follows:

-- Synthesis of [NO2-tyrosine-BSA] NO_2-tyrosine-BSA : The synthesis of this conjugate requires 20 mg of the NO_2-Tyr (Sigma) hapten and 20 mg of BSA. [Le] The coupling takes place with carbodiimide following the same protocol described above.--

Please replace the paragraph beginning at page 26, line 1, with the following rewritten paragraph:

-- The polyclonal serums were adsorbed on the corresponding non-nitrolysated conjugates: Tyr-BSA/HSA for the "T" rabbit and Cys-BSA/HSA for the "C" rabbit. (Geffard et al., 1984a; Geffard et al., 1985b; Campistron et al., 1986). The adsorption took place in proportions of 5 mg of conjugate per ml of pure serum. The mixture was incubated for 16 hours at 4°C under agitation and the immunoprecipitates were eliminated by centrifugation for 15 minutes at [10 OOOg] 10000g. [Le] The supernatant is enriched in specific Ig, while the pellet contains the rabbit Ig-carrier protein immune complexes.--

Paragraph beginning at page 26, line 10, has been amended as follows:

-- To one volume of rabbit polyclonal serum an equal volume of a saturated ammonium sulfate solution $(NH4)_2SO_4$ is added. The mixture was incubated for 1 hour at 4°C, and then centrifuged

for 15 minutes at [10 OOOg] 10000g. The cell (containing the Ig precipitates) is taken up in a minimum volume of TPB buffer and then dialyzed for 3 days in a SPB buffer (Na_2HPO_4, 0.01 M, NaCl 0.15 M).--

Paragraph beginning at page 30, line 24, has been amended as follows:

-- Decrease in the OD (B) indicates the presence of competition between the conjugated hapten which is adsorbed on the microtitration plate and the hapten preincubated with the corresponding antiserum. Bo is the OD corresponding to the response obtained with the antiserum in the absence of the competitor. A dilution of the antiserum (1/20,000) yielding a OD of approximately 1.0 [à] to 492 nm was chosen for adjustment of the value of Bo; the B/Bo relationship was used to trace the competition curves of figure 5 obtained with the competitors.--

Paragraph beginning at page 43, line 8, has been amended as follows:

-- Despite the absence of competition with NO-BSA, indirect ELISA tests demonstrated that the monoclonal Ab recognizes the nitrosylated protein. The supernatant (1/5) and ascite fluid (1/30,000) respectively yielded OD of 0.36 ± 0.085 [et] and 0.54 ± 0.12. The results represent the average and standard deviation of three experiments.--

Last line on page 50 (line 32), has been amended as follows:

-- -NO-Cys-BSA [et]and NO-Cys-G-BSA: --

APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

Paragraph beginning at page 61, line 25, has been amended as follows:

--The serums of the ten rats drawn for 5 weeks were tested on conjugates NO-Cys-G-BSA; NO-Tyr-BSA; [N02-Tyr-BSA] NO_2-Tyr-BSA, and on the corresponding non-nitrosylated conjugates. Figures 26 and 27 show the "Control" and "NIS" groups, respectively, with the progression over time of the antibodies induced against two epitopes: [N02-Tyr-BSA] NO_2-Tyr-BSA and NO-Tyr-BSA. These results represent the average on two tests. The OD obtained in each group are equivalent on all conjugates tested, each point represents the average and the standard deviation of the OD obtained with the 5 rats in the same group for the same conjugate.--

Paragraph beginning at page 54, line 3, has been amended as follows:

-- - Recent work has shown the formation of nitrotyrosines at inflammatory sites (Kaur and Halliwell, 1994). To detect the presence of immunological responses to these epitopes in the serums of rats, [NO_2-Tyr-BSA] NO_2-Tyr-BSA and the conjugated nitrosotyrorine (NO-Tyr-BSA) were used. Tyr-BSA was used to correct OD obtained on : [NO_2-Tyr-BSA] NO_2-Tyr-BSA and NO-Tyr-BSA.--

Paragraph beginning at page 62, line 12, has been amended as follows:

-- For "Aminoguanidine": (Figure 28) anti-NO-Tyr is the highest response; the OD are between 1.5 [et] and 2 (between the 2nd and 5th weeks). The anti-N02-Tyr response is as large; note an increase in signals between the 1st and 2nd weeks. They stabilize until the 3rd week, then increase slightly toward the 4th week.--

In the claims:

Claims 1-5, 11 and 14 have been amended as follows:

APPLICANTS: Chagnaud *et al.*
U.S.S.N.: 09/331,980

1. (Amended) A purified antibody [recognizing] , wherein the antibody binds specifically to a nitrosylated protein.

2. (Amended) The antibody [in] of Claim 1, [characterized by the fact that said] wherein the nitrosylated protein is a transporter of NO.

3. (Twice amended) The antibody [in] of Claim 1, [recognizing] wherein the antibody binds specifically to a nitrosylated albumin.

4. (Twice amended) The antibody [in] of Claim 1, [characterized by the fact that it] wherein the antibody is a polyclonal antibody.

5. (Twice amended) The antibody [in] of Claim 1, [characterized by the fact that it] wherein the antibody is a monoclonal antibody.

11. (Twice amended) A pharmaceutical [compound] composition, [characterized by the fact that it contains as its active ingredient] comprising:

(a) a purified [an] antibody [according to Claim 1 advantageously dispersed in] that binds specifically to a nitrosylated protein; and

(b) a pharmaceutically acceptable [vehicle or] excipient[s].

APPLICANTS: **Chagnaud *et al.***
U.S.S.N.: **09/331,980**

14. (Twice amended) A kit for [use of the process in Claim 13] *in vitro* detection of nitrosylated proteins in a biological specimen, [characterized by the fact that it contains] comprising :

(a) [at least one antibody according to Claim 1 which may be marked] a purified antibody that binds specifically to a nitrosylated protein, and

(b) reagents to produce a medium favorable for an immunological reaction between said purified antibody and any nitrosylated proteins that may be present in a biological specimen. [potentially one or more reagents for detection, which may be marked and can react with any immunological complexes that may be formed, potentially one or more biologic reagents for reference and control].